ologic conditions of the eggs which these investigators used. These variations have now been adequately described and measured, and correlated with physiologic condition of the eggs. For experimental work it is absolutely necessary to determine in advance the exact physiologic condition of the eggs, and to use only such, as are nearly in the same condition. We may then hope to obtain more constant and predictable results.

A full account of these experiments will appear in the forthcoming volume of the Researches of the Marine Biological Laboratory of the Carnegie Institution of Washington.

TRANSPLANTATION OF LIMBS

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The specificity of the tissue of the limb bud in the amphibian embryo has been clearly shown by Braus,[1] who found that when transplanted to any part of the body it would develop into a normal appendage. Since then evidence has accumulated[2] to show that the limb rudiment, and more especially its mesoderm, constitutes an equipotential system.[3] Legs are, nevertheless, rights or lefts and, having no plane or axis of symmetry, the leg of one side of the body cannot be superimposed upon that of the other. In the early embryonic condition, however, there is no visible evidence of laterality, and the question arises when and how this property is determined. Experiments made during the past year have rendered it possible to state more simply than before[4] the rules that govern its determination.

All of the experiments here considered were made with the fore limb of Amblystoma punctatum under precautions necessary to prevent regeneration from the host.[2] In grafting the limb buds three different circumstances relating to their position in the embryo were taken into account—location, laterality and orientation (fig. 1). First, the limb buds were placed either in their natural location in another embryo after removal of the normal bud (orthotopic transplantation) or else in some other region of the body, preferably on the flank of the embryo between the normal fore and hind limbs (heterotopic transplantation). Secondly, some were grafted on the same side of the body as that from which they were taken (homopleural) and others on the opposite side (heteropleural). Thirdly, they were placed either in upright position, with the dorsal border of the transplanted disc corre-

sponding to the dorsal border of the wound (dorso-dorsal), or in the inverted position with the ventral border of the disc at the dorsal border of the wound (dorso-ventral). There were thus eight different possible combinations, all of which were tried in numerous individual cases. Intermediate positions have not yet been experimented with. When the limb bud of one side is implanted on the other, it is obviously impossible to place it in a normal posture, for when the dorsal and

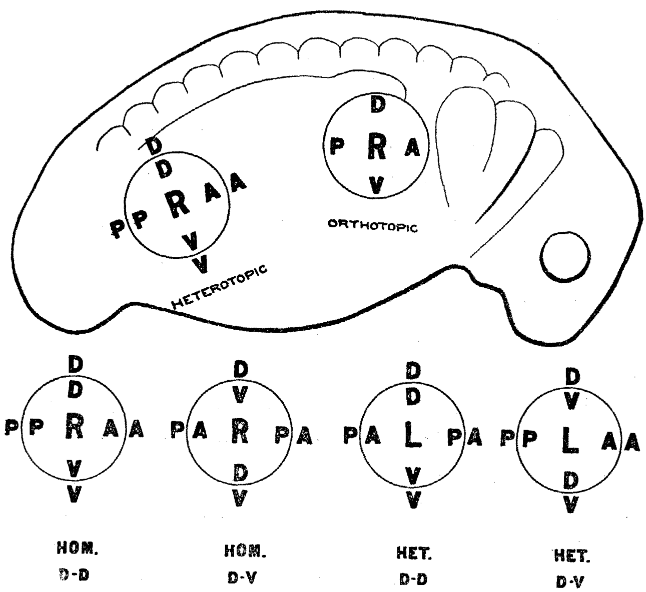

FIG. 1.

Diagram showing the eight different operations. The outline of an Amblystoma embryo in the operating stage is shown above. The circles within it represent the limb bud, in the normal (orthotopic) and the abnormal (heterotopic) location. The four circles below represent the four different ways in which limb buds may be oriented with reference to the cardinal points of the embryo; the letters (*d*, dorsal; *v*, ventral; *a*, anterior; and *p*, posterior) within the circles designate the original cardinal points of the transplanted limb, those outside the corresponding points of the embryo. The operations are represented to be on the right side. *R*, right limb bud; *L*, left limb bud; *hom.*, homopleural; *het.*, heteropleural.

ventral borders correspond respectively the anterior and posterior borders are interchanged and vice versa. Eight further combinations are formally possible by interchange of internal (medial) and external (lateral) surfaces, though they are impracticable, because the mesoderm would thereby be placed on the outside.

The experiments confirm previous ones in showing that the limb bud is a self differentiating body, in so far as it may produce a fore limb

wherever or in whatever position implanted, but they also show that its laterality may be affected by its new surroundings. There are many instances of imperfect development, and of total absorption of the transplanted tissue, especially in the case of the heterotopic transplantations. Also, as has been observed before (Braus,[5] Harrison,[6]) supernumerary appendages often arise after transplantation, just as in regenerating limbs under certain conditions (Tornier,[7] Della Valle.)[8]

In the orthotopic operations the transplanted limb, whatever its form, usually becomes functional, while in the heterotopic this is rarely the case and the function is never perfect. Functional activity, together with the superior potency of the norma surroundings in influencing the development of the limb, renders the results of orthotopic transplantation, in so far as they bear on the problem of laterality, more complicated and difficult to interpret than the heterotopic. Nevertheless the following fundamenta rules underlie the determination of laterality in both cases.

Rule 1. A bud that is not inverted (dorso-dorsal) retains its original laterality whether implanted on the same or on the opposite side of the body.

Rule 2. An inverted bud (dorso-ventral) has its laterality reversed whether implanted on the same or on the opposite side.

Rule 3. When double or twin limbs arise the original one (the one first to begin its development) has its laterality fixed in accordance with the above rules, while the other is the mirror image of the first.

In the heterotopic transplantations the tissue is very often resorbed or the appendages which arise are rudimentary or imperfect (56%). Probably vascularization is not so good and innervation is incomplete. Reduplications were produced in all of the four combinations.

In the orthotopic transplantations only about 18% of the limbs were rudimentary or imperfect, the rest being either fully developed reduplications or normal limbs. Double limbs were obtained frequently in two of the combinations (heteropleural dorso-dorsal and homopleural dorso-ventral) and were very rare in the other two. Other modifications also occurred. The results were as follows:

1. Homopleural dorso-dorsal grafts developed normally though at first very slightly retarded. Rule 1; 6 cases (100%).[9]

2. Homopleural dorso-ventral grafts resulted in (*a*) A single limb of reversed laterality (a structurally and functionally perfect right limb on the left side). Rule 2; one case only (4.3%). (*b*) Reduplicated limbs. Rule 3; 16 cases (69.6%). (c) Typical non reversed limbs which began their development by growing in an abnormal direction,

but ultimately assumed normal posture by rotation. 6 cases (26%). The latter, which constitute the only exception to the rules, require further investigation.

3. Heteropleural dorso-dorsal transplantations yielded: (*a*) Single non reversed limbs. Rule 1; one case only, and that not perfect (3%).

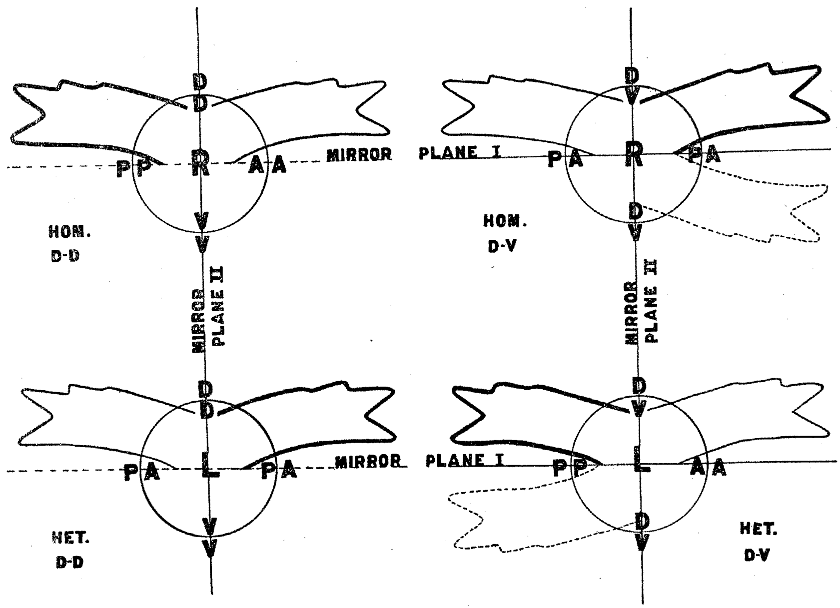

FIG. 2.

Diagram showing the results of the four operations, heterotopic or orthotopic, represented as on the right side of the embryo. The circles indicate the transplanted limb buds, the letters having the same significance as in figure 1. Thus the two upper figures in the diagram represent homopleural, and the two lower ones heteropleural transplantations. The two on the left show the transplanted bud in upright (dorso-dorsal) orientation while the two on the left are inverted (dorso-ventral). The limbs which develop are shown in profile, the ulnar border being uppermost (dorsal) in all which actually develop. A heavy outline indicates the primary member, a light outline the reduplicating one. It is to be noted, however, that the latter develop in by no means all cases while the former may be resorbed in the heteropleural dorso-ventral combination leaving only the reduplicating member present. The broken outlines show the posture that the limb would have assumed, had it developed as a self differentiating member totally independent of the influence of its surroundings.

(*b*) Reduplicated limbs, in which the secondary member, being reversed, has the laterality of its new surroundings. Rule 3; 26 cases (78.8%). (*c*) Cases similar to the above (*b*) in their early development but differing later in that the reduplicating bud gained the upper hand and developed into a normal functioning limb of reversed laterality

(corresponding to its new surroundings); at the same time the original bud became reduced to a small spur or appendage upon the other. Rule 3 modified; 6 cases (18.2%).

4. Heteropleural dorso-ventral transplantations developed into: (*a*) Single limbs of reversed laterality somewhat retarded in their development. Rule 2; 17 cases (94.4%). (*b*) Duplicate limbs. Rule 3; a single case only (5.5%).

The results of the experiments are summed up in diagrammatic form in figure 2. The ulnar border of the limb is at first always dorsal slightly inclined to medial, as in the normal appendage, while the palmar surface is medial slightly inclined to ventral, but the limb may point in either an anterior or a posterior direction. Bending of the elbow or rotation from the shoulder joint may of course modify the positions later in development. The reversal of laterality which takes place is as if the limbs were mirrored in either one of two planes, one of which is horizontal and the other vertical with respect to the main axis of the embryo. When the reversal takes place across the former plane it is complete and no reduplication occurs. It is accomplished immediately or at least very early in development, so that there is little or no outward sign of disturbance and development is but slightly retarded. This plane may be called the primary one. It comes into play when the dorso-ventral axis of the transplanted bud is inverted. When mirroring takes place in the vertical plane, which may be called the secondary one, actual reduplications are found. This occurs in those combinations in which the dorso-ventral axis of the disc is not inverted and it may also occur secondarily following reversal across the primary plane as just described. The twinning of the appendages may, however, be masked, at least in orthotopic transplantations, through the absorption of the original (primary) limb bud (3 c).

The relations of the duplicate limbs change considerably during development, and their definitive position is subject to considerable variation. There may be further reduplication, so that more or less complete triple limbs may result. The three limbs then have approximately the same relations as found by Bateson,[10] especially in arthropods.

In two of the combinations the limbs that develop are in normal orientation with respect to the cardinal points of the embryo. One of these (hom. dd.) is when the graft is placed in the original normal posture, the other (het. dv.) when it is placed upside down on the opposite side of the body. These combinations may be termed harmonic. The other two combinations, the simply inverted bud (hom. dv.) and the bud planted on the opposite side of the body in upright position (het.

dd.), result primarily in limbs that are not normally placed in relation to the cardinal points of the embryo, and may be termed disharmonic.

In comparing the distribution of single and reduplicated appendages in the various groups a remarkable result becomes apparent. In the heterotopic group, where function is excluded, the harmonic combinations yield a relatively large proportion of reduplications (about 54%), while the disharmonic combinations yield a far greater proportion of single limbs (about 87%). In the orthotopic group, on the other hand, the harmonic combinations yield in overwhelming proportion (96%) single limbs which are functional and perfectly normal with respect to their surroundings, while the disharmonic combinations yield about 96% of reduplications.

Experiments with superimposed limb buds and with transplanted half-buds confirm the above results. The harmonic combinations yield single limbs while the disharmonic combinations result in reduplications.

All double formations do not remain as such, however, for it is possible to achieve a normal result from at least one of the disharmonic combinations (het. dd.) by the reduction of the original limb of the pair and the preponderance of the other, which then becomes a normal single limb of reversed laterality (3c) corresponding, therefore, to the laterality of its new surroundings. On the other hand, in the only case in which a single normal limb of opposite laterality (2a) developed in the orthotopic position, it functioned perfectly. This shows that when innervation and vascularization are sufficient, a functional condition may arise which is independent of the harmony of the combination.

It would be premature to discuss the bearing of the experiments upon the question of adaptation. It may be pointed out, however, that the fundamental rules of laterality, as stated above, lead as often to disharmonic as to harmonic results. On the other hand the secondary factors, especially those which determine whether a reduplicated or a single appendage shall arise and those which lead to the resorption of one of the two members of a pair, show a tendency to produce a preponderance of adaptive results. To determine the exact nature of these factors will require further investigation, pending which it seems unwise to appeal to mysticism for the explanation.

[1] Braus, H., *Münchener med. Wochenschr.*, 1903.

[2] Harrison, R. G., these PROCEEDINGS, **1**, 1915.

[3] The shoulder girdle rudiment is itself such a system according to Braus, *Experimentelle Beiträge zur Morphologie*, **1**, 1909 (400). Also *Morph. Jahrb.*, *Leipzig*, **39**, (271).

[4] Harrison, R. G., *Anat. Rec.*, *Philadelphia*, **10**, 1916.

[5] Braus, H., *Anat. Anz., Jena*, **26**, 1905, (461).
[6] Harrison, R. G., *J. Exp. Zool., Philadelphia*, **4**, 1907, (254).
[7] Tornier, G., *Arch. Ent.-Mech., Leipzig*, **20**, 1905.
[8] Valle, P. della, *Napoli, Boll. Soc. Nat.*, **25**, (1911–12), 1913.
[9] In calculating percentages only those cases which yielded positive results were taken into account.
[10] Bateson, W., *Materials for the Study of Variation*, London, 1894, (479).

THE SHAPES OF GROUP MOLECULES FORMING THE SURFACES OF LIQUIDS

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In a recent paper[1] I have developed a theory according to which all the forces involved in the structure of solids and liquids, are similar in nature to the forces causing chemical combination. Thus condensation, evaporation, adsorption, cohesion, crystallization, liquefaction, viscosity, surface tension, etc., are manifestations of the forces already known to the chemist. In all these cases the range of the forces is limited to atomic dimensions except in so far as their effects may be transmitted from atom to atom. According to this theory, every atom in a solid or liquid is chemically combined to every adjacent atom. This chemical union may be strong or weak and may be characterized either by primary or secondary valence (Werner).

In most inorganic solid or liquid substances of the strongly polar type, the identity of the molecule is wholly lost, but in organic compounds the groups of atoms constituting the chemical molecule usually have a real existence even in the liquid or solid state. These group molecules are held together by primary valence forces while the forces acting between the group molecules, although no less chemical than the others, are to be characterized as secondary valence forces.

From this viewpoint the forces involved in adsorption and surface tension do not originate from the group molecule as a whole, but rather from certain atoms in the molecule.

This theory leads inevitably to the conclusion that adsorbed films on plane surfaces of solids or liquids should, in general, be one atom or group molecule in thickness. Considerable experimental evidence has already been presented that this is the case with films of gases adsorbed on solids.

Miss A. Pockels[2] showed, in 1891, that very small amounts of oil on the surface of water have no appreciable effect on the surface tension,